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BIOLOGICAL NANOPLATFORMS FOR SELF-ASSEMBLED ELECTRONICS

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1. SUMMARY

Amino acid sequences that display the ability to bind to different metal substrates have been found by phage display library. Virus-like particles (VLPs) are protein shells that are capable of presenting multiple copies of single amino acid sequences through chemical modification of the VLP surface. This report describes efforts to assign gallium arsenide binding abilities to VLPs from the bacteriophage MS2 by chemically linking artificial metal binding peptides to the VLPs' surfaces, then confirm the ability of the VLPs to bind gallium arsenide.

2. INTRODUCTION

Over the last several years the field of phage display libraries has increased researchers' abilities to rapidly discover amino acid sequences that bind to targets of interest [1]. Phage display libraries work by inserting random amino acid sequences into the gene of the coat protein of a virus such as M13, a virus that infects *Escherichia coli*. Approximately one billion different amino acid sequences are displayed on different viruses in the same pool of viruses [1]. A substrate of interest, such as a pure metal surface, is exposed to the pool of viruses. A small subset of the viruses binds to the substrate through the amino acid sequences engineered into their coat proteins. Those viruses that bind are collected, purified, then the process is repeated with the binding viruses to find those with the highest affinity for the substrate. This is called panning, like gold panning. The viruses with highest affinity then have their coat protein sequences determined, which reveals the random sequence that generated binding to the substrate. This random amino acid sequence is the equivalent of antibodies generated by animals. That sequence can then be generated chemically to yield a short string of amino acids that hopefully retain their ability to bind to the target substrate. The short string can be chemically linked to a number of materials to confer binding specificity to the materials.

Virus-like particles, or VLPs for short, are the shell of viruses that enclose their genetic material. They are comprised of coat proteins, and are assemblies that contain conserved numbers of coat proteins. Bacteriophages, viruses that infect bacteria, generate VLPs that are very stable in a variety of conditions. The amino acid sequences, and corresponding three-dimensional structures, of many bacteriophage VLPs are known. Knowledge of the positions of individual amino acids within the three-dimensional structure of the VLP allows researchers to use specific amino acids, such as tryptophan, as sites to which ligands can be bound to the surface of VLPs. This approach is being used by a number of laboratories as a platform for new classes of vaccines against many diseases. In a materials science context, it may also be possible to create VLPs that are able to bind to different metal substrates. Such functionalized VLPs could serve as connections in molecular circuits. The goal of this project was to fuse gallium arsenide-binding peptides (as shown by phage display) to bacteriophage MS2 coat proteins, then assess their ability to bind to gallium arsenide substrates.

3 METHODS, ASSUMPTIONS, AND PROCEDURES

3.1 Peptide binding to VLPs

Phage MS2 VLPs at a storage concentration of 1.5 mg/ml were diluted into a 1X phosphate buffered saline (PBS) solution to a final volume of 100 microliters (μl). SMPH was added to the reaction mixture, then incubated for one hour at room temperature. Unbound succinimidyl-6-(β-maleimidopropionamido)-hexanoate (SMPH) was then removed from the VLPs by filtration with Amicon Ultra filters, 100 kilodalton (kDa) cutoff. Modified VLPs were resuspended in 1X PBS. Peptides were added to the VLPs at a 20 fold excess of peptide to binding site, then incubated for 1 hour at room temperature.

3.2 Purification of modified VLPs

Upon completion of binding reactions, peptide-VLP reaction mixtures were passed through Amicon Ultra filters, 100 kDa cutoff. The retentate was resuspended in 100 μl 1x PBS, then stored at 4 degrees C.

3.3 Verification of peptide-VLP binding

5-20 μl of reaction mixture was applied to polyacrylamide gels (BioRad Laboratories), then electrophoresed until the leading dye band of the loading buffer reached the bottom of the gel. Band detection occurred using Coomassie Blue stain (BioRad Laboratories). Gel images were archived using a GelDoc EZ imaging system (BioRad Laboratories).

3.4 Binding of modified VLPs to substrates

The binding reaction between modified VLPs and control or targeted substrates occurred by incubating the substrate in solution containing modified VLPs. Short incubation reactions (two hours or less) occurred at room temperature. Long incubation reactions (one to seven days) took place at 4 degrees C in a sealed, humidified cold box.

3.5 Verification of modified VLP binding to substrates

Potential modified substrates were washed by immersion in 18.2 megaohm (MOhm) water, then wicked dry. VLP contrasting consisted of using 2% uranyl acetate to stain the modified surface. After two minutes the stain was wicked off and the grids air-dried. The substrates were imaged in a Hitachi H7500 transmission electron microscope (TEM) operating in high contrast mode. Images were collected with an Advanced Microscopy Techniqiues (AMT) XR60 bottom mount camera.

4 RESULTS AND DISCUSSION

Our goal was to generate gallium arsenide surfaces decorated with VLPs. VLP adhesion to GaAs was to occur by modifying the VLPs with peptides shown by phage display to bind GaAs [2], [3], [4]. Previous studies into modification of VLPs with gold-binding peptides took advantage of colloidal gold nanoparticles to visualize individual binding events. These peptides showed high binding affinity due to their modification with thiol-containing amino acids such as cysteine. The gold-thiol interaction results in the formation of a covalent bond, thereby aiding in retention of gold nanoparticles. Since gallium arsenide nanoparticles do not exist, a different assessment protocol was needed. Thin films of GaAs were deposited on 8 nanometer (nm) silicon nitride films. It was hypothesized that the thin GaAs films would retain the ability to react with GaAs-binding peptides, while still allowing analysis by TEM [5].

Figure 1 shows an image of binding characterization reaction. Phage MS2 has three potential sites for peptide binding. Perfect modification reactions result in all three sites being occupied by modifying peptides. In reality the stoichiometry of binding is different at all three sites due to steric and ionic influences from the topology of the phage coat protein. The electrophoretic data shows four bands, each one due to a different population of coat protein. The bands closest to the bottom of the image are unmodified coat protein. Coat proteins modified by one, two or three peptides, respectively, form the bands immediately above the bottom band. Bands occurring higher in the gel are multimers of the coat protein, and also display peptide modification patterns. These results show us that the majority of the coat proteins contain at least one modification peptide. Thus, each VLP, which contains 180 coat proteins, displays between 180 and 540 modifying peptides on its surface (1-3 peptides per coat protein x 180 coat proteins per VLP shell).

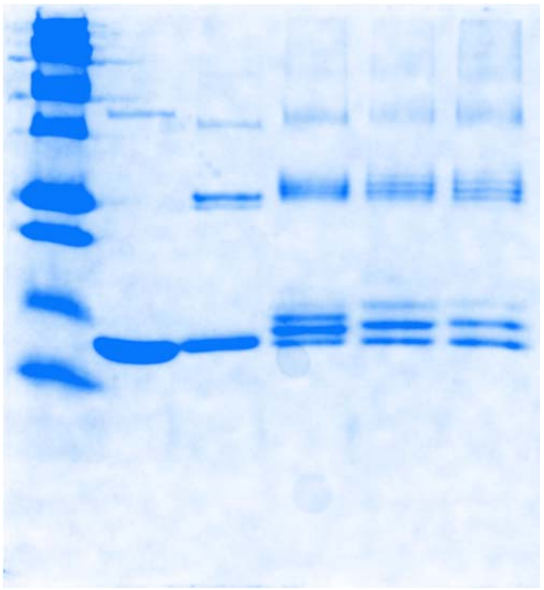

Figure 1: modification of phage MS2 coat protein with metal binding peptides.

A large number of sites containing peptides that bind to GaAs exist on the surface of each VLP. However, successful binding was shown for the sequence when contained within a phage M13 coat protein sequence, not chemically linked to the surface of phage MS2 VLPs. Thus, binding properties may change when the peptide is presented in a different three dimensional environment.

Transmission electron microscopy (TEM) served as the analysis method to assess binding of modified VLPs to GaAs [5]. Figure 2 shows modified VLPs electrostatically bound to thin (8 nm) thick carbon films. The size bar in the lower right corner of the image denotes a distance of 100 nm in the image. Chemical linking of the GaAs binding peptides to the MS2 coat protein surface did not cause morphological changes in the structure of the VLP. The radius of the modified VLPs is 28 nm, the same as that measured for wild type phage MS2. However, upon incubation of modified VLPs on GaAs thin films, no binding events were recorded. Different incubation times were used, but the GaAs surfaces appeared refractive to phage binding, or the modified phages were unable to maintain stable binding to the substrates under the experimental conditions.

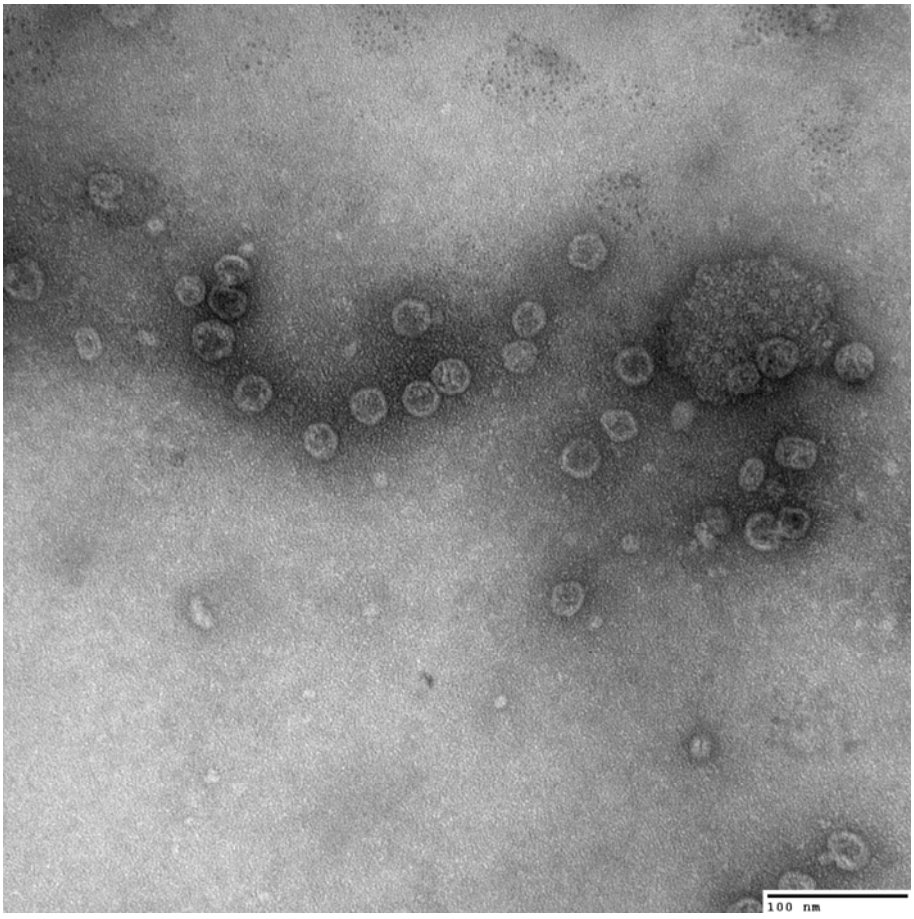

Figure 2: Modified VLPs retain the dimensions and shape of unmodified VLPs.

5 CONCLUSIONS

It is a dangerous assumption that a peptide which shows a specific binding activity in the context of a phage display binding assay will show the same activity in a different context. This has been the case with the gallium arsenide binding peptides. While it is possible to decorate the surface of bacteriophage MS2 with multiple peptides that have shown the ability to binding gallium arsenide in a bacteriophage M13 phage display library, MS2 VLPs modified with the metal binding peptides do not display the same activity. This is most likely due to change in the 3D conformation of the peptides as they are presented on the MS2 VLP surface.

It is proposed that a new panning experiment be performed to look for amino acid sequences that bind to gallium arsenide. However, in this new experiment the MS2 phage display library developed by Dr. David Peabody at the University of New Mexico Health Sciences Center would be used [6] rather than a traditional M13 phage display system. The use of Dr. Peabody's system would obviate the need to chemically modify wild-type VLPs with metal binding peptides, as the metal binding sequence would be contained within the amino acid sequence of the coat proteins. Native VLPs that show metal binding abilities would subsequently be used for further surface modification studies, thereby ensuring the VLPs used are active for metal binding activity.

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LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

AMT	Advanced Microscopy Techniques
GaAs	gallium arsenide
kDa	kilodaltons
M13	bacteriophage M13
MOhm	megaohm
MS2	bacteriophage MS2
nm	nanometer
PBS	phosphate buffered saline
peptide	a sequence of amino acids
phage	bacteriophage
SMPH	succinimidyl-6-(β-maleimidopropionamido-hexanoate
TEM	transmission electron microscopy
VLP	virus-like particle
ųl	microliter

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